CaM is RyR2 bound. These results are the first quantitative in situ RyR2-CaM binding measurements.

2239-Pos Board B225
A Region Involved in Isoform-Specific Regulation of Skeletal Muscle Ryanodine Receptor by Calmodulin
Naohiro Yamaguchi, Le Xu, Gerhard Meissner.
Calmodulin (CaM) regulates both skeletal (RyR1) and cardiac (RyR2) muscle ryanodine receptor calcium channels by binding to a single highly conserved CaM binding domain (RyR1 amino acid (aa) 3614-3643; RyR2 aa 3581-3610). CaM inhibits both isoforms at an elevated Ca^{2+} concentration (micromollar), whereas at cellular resting Ca^{2+} concentration CaM has opposite effects on two isoforms (activation of RyR1 and inhibition of RyR2). This implies another region of RyR is involved in isoform-specific CaM regulation at submicromollar Ca^{2+} concentration. To identify the regions we constructed and analyzed a series of RyR1/RyR2 chimera. RyR1/RyR2 chimera carrying RyR1 aa 1-3725 is inhibited by CaM at 0.4 μM Ca^{2+} (RyR2-type), whereas chimera carrying RyR1 aa 1-4301 is activated (RyR1-type). The results suggest that RyR1 aa 3726-4301 contains a region that is responsible for CaM activation. The region overlaps with a domain resembling the sequence of CaM (CaM-like domain). Replacement of RyR1 3726-4301 with the corresponding RyR2 sequence confers CaM inhibition at $[Ca^{2+}]<1$ μM (RyR2 type). Furthermore, substitution of 5 non-conserved amino acids in RyR1 CaM-like domain with those of RyR2 (M4122T, I4123L, N4124D, F4125Y, N4130K) is sufficient for RyR2-type CaM inhibition. However, the reverse chimera and mutant RyR2 were not activated but inhibited by CaM. Taken together, the results suggest that 5 non-conserved amino acids of RyR1 are crucial for RyR1-specific CaM activation at submicromollar Ca^{2+} concentration. On the other hand, CaM inhibition of RyR2 is likely controlled by a different region. Supported by NIH (AR018687 and HL073051), NSF (EPS-0903795) and AHA (10SDG3500001).

2240-Pos Board B226
Effect of Human RyR2 CPVT Mutations on Interaction with Calmodulin
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The ryanodine receptor (RyR) is a member of a family of intracellular calcium release channels that regulate calcium efflux from intracellular stores. The RyR2 isoform is most abundant in the heart and plays a key role in cardiac muscle excitation-contraction coupling. Clusters of mutations associated with the inherited arrhythmogenic disorder, catecholaminergic polymorphic ventricular tachycardia (CPVT), have been found in specific regions throughout RyR2, a large protein of ~5000 amino acids. Many of these CPVT mutations (total >100) are thought to occur in significant functional domains and result in the dysregulation of RyR channel function.
One such region of RyR2 is believed to comprise a calmodulin (CaM) interaction site and two EF hand motifs. The calcium-sensitive binding of CaM has been shown to regulate the opening of RyR. Hence, examining the RyR2 interaction with CaM and the potential effects of CPVT mutations on this binding may help reveal mutation-dependent mechanisms of channel dysfunction.
We have prepared bacterial expression plasmid constructs containing the wild-type human RyR2 CaM-interacting domain and introduced a series of CPVT mutations that have previously been identified to occur within this region. Expression and purification of the corresponding recombinant fusion proteins has enabled calcium-dependent binding of CaM to be determined with all these constructs. Examining the distinct functional role of calcium concentration on CaM binding kinetics and further comparative structural analyses of the wild-type and mutant RyR2 domains may help reveal the specific effect(s) that CaM-mediated regulation may have in mediating CPVT-linked arrhythmogenesis.

2241-Pos Board B227
Direct Detection of Domain Peptide Binding to the Cardiac Ryanodine Receptor (RYR2) using FRET
Florentin R. Nitu, Razvan L. Cornea, David D. Thomas, **Bradley R. Fruen**.
The RyR2 Ca release channel is activated by the synthetic domain peptide DPc10, which corresponds to a 36-residue sequence within the channel's central mutation hot-spot region. DPc10 activation is hypothesized to result from the destabilization of a critical intramolecular interaction between the N-terminal and central hot-spot regions of the full-length RyR2, and to thereby mimic the effects of arrhythmogenic mutations on this putative intramolecular interaction controlling channel activation. However, the site of DPc10 binding within the RyR2 3D structure is uncertain, and factors that may modulate binding are undefined. To directly monitor and map DPc10 binding to the RyR2, we attached a FRET acceptor at DPc10's N-terminus. A FRET donor was targeted to the RyR2 cytoplasmic assembly via a fluorescent-labeled FKBP12.6. Addition of the acceptor-labeled DPc10 (30 μM) resulted in a marked decrease in fluorescence of the RyR2-bound FKBP12.6. Fluorescence was partially restored upon FKBP12.6 dissociation from the RyR2, indicating that a major fraction of the total fluorescence decrease was attributable to FRET between FKBP12.6 and DPc10 when bound to the channel. The DPc10 dependence of FRET was similar to the DPc10 dependence of RyR2 activation observed previously in bilayer and ryanodine binding studies (EC_{50} ~25 μM), consistent with the likelihood that FRET reflected DPc10 binding at its regulatory site on the RyR2. FRET decreased as a function of increasing Ca (30 nM to 300 μM), suggesting that Ca activation of the RyR2 altered either the affinity of DPc10 binding or its proximity to the FKBP12.6 subunit. We conclude that DPc10 binds to a site on the RyR2 within 10 nm of FKBP12.6. Regulatory interactions and structural changes at this site can be monitored using FRET.

2242-Pos Board B228
β Strand Switching: A Novel Structural Rescue Mechanism in a Δexon3 Cardiac Ryanodine Receptor Mutant
Paolo A. Lobo, Lynn Kimlicka, Ching-Chieh Tung, Filip Van Petegem.
The contraction of cardiac muscle requires release of Ca2+ from the sarcoplasmic reticulum through the cardiac ryanodine receptor (RyR2). Several mutations in RyR2 are linked to inherited disorders, including triggered cardiac arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT) that may lead to sudden cardiac death. A severe form of CPVT is caused by removal of an entire third exon (Δexon3) of RyR2. The 35 deleted residues form secondary structure elements which are crucial in folding of the N-terminal domain, raising the question of why the deletion is neither lethal nor confers a loss-of-function phenotype. A 2.3Å crystal structure shows that the removal results in a structural rescue: an otherwise flexible loop compensates for the loss by inserting itself into the β trefoil domain and increases the thermal stability. The other β strands in the domain show increased mobility to accommodate a sequence that bears no similarity to the deleted exon. The exon3 deletion is not tolerated in the corresponding RyR1 domain. The rescue shows a novel mechanism by which RyR2 channels can adjust their Ca2+ release properties through altering the structure of an individual domain.

2243-Pos Board B229
Crystallographic Investigation of Several Malignant Hyperthermia and CPVT Mutations in Ryanodine Receptors
Lynn Kimlicka, Filip Van Petegem.
Mutations in the Ryanodine Receptor (RYR) are known to underlie many genetic diseases. In particular, the skeletal muscle isoform (RyR1) is involved in malignant hyperthermia (MH) and central core disease (CCD), whereas mutations in the cardiac isoform (RyR2) are known to cause catecholaminergic polymorphic ventricular tachycardia (CPVT) and arrhythmogenic right ventricular dysplasia (ARVD). Despite an extensive analysis of disease mutations on the functional level, very little is known about the structural changes induced by the mutations. Here we analyze the structures and stability of over 10 different disease mutants. We present crystal structures of mutant versions of the RyR1 N-terminal disease hot spot, encoding three domains, and of the RyR2 N-terminal domain, all solved between 2.0 and 3.0 Angstrom, and compare them to the wild type structures. The observed effects on structure and stability differ substantially among the mutants. Whereas some cause a major destabilization of the overall fold, others mainly cause relative domain-domain movements or confer large conformational changes within individual domains. We discuss the likely implications of the disease mutations on the overall structure and gating properties of the intact RyR.

2244-Pos Board B230
A Model-Based Description and Burst Analysis of Purified Human Cardiac Ryanodine Receptor (hRyR2) Gating Kinetics Under Minimal Conditions
Saptarshi Mukherjee, Nia Lowri Thomas, Chloe E. Maxwell, Alan J. Williams.
Rhythmic contraction of cardiac myocytes is maintained by precisely controlled Ca^{2+} efflux from intracellular stores mediated by the cardiac ryanodine receptor (RyR2). Mutations in RyR2 can cause channel instability leading to perturbed Ca^{2+} release that can trigger arrhythmias. RyR2-dependent ventricular tachyarrhythmia is an important cause of sudden cardiac death, the mechanistic basis of which remains unclear. Most investigations of RyR2 single channel function have focussed on the secondary effects of mutations on channel function through modulation by regulatory proteins and cellular processes without emphasis on mutation-dependent effects on the gating behaviour of the channel itself. Here we describe the gating kinetics of wild-type hRyR2 when activated by its physiological trigger, cytosolic Ca^{2+} and isolated from the possible modulatory effects of regulatory factors external to the channel. Single channel experiments were performed using recombinant, purified hRyR2 under reducing conditions where the luminal Ca^{2+} was buffered at 50 nM while the cytosolic Ca^{2+} was stringently controlled using EGTA, HEDTA and NTA to achieve an activating